Extensive Disease Small Cell Lung Cancer Dose-Response Relationships

Implications for Resistance Mechanisms

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Background: Some studies (but not others) suggested that high doses are beneficial in small cell lung cancer (SCLC). We hypothesized that dose-response curve (DRC) shape reflects resistance mechanisms.

Methods: We reviewed published SCLC clinical trials and converted response rates into estimated mean tumor cell kill, assuming killing is proportional to reduction in tumor volume. Mean % cell survival was plotted versus planned dose intensity. Nonlinear and linear meta-regression analyses (weighted according to the number of patients in each study) were used to assess DRC characteristics.

Results: Although associations between dose and cell survival were not statistically significant, DRCs sloped downward for five of seven agents across all doses and for all seven when lowest doses were excluded. Maximum mean cell kill across all drugs and doses was approximately 90%, suggesting that there may be a maximum achievable tumor cell kill irrespective of number of agents or drug doses.

Conclusions: Downward DRC slopes suggest that maintaining relatively high doses may possibly maximize palliation, although the associations between dose and slope did not achieve statistical significance, and slopes for most drugs tended to be shallow. DRC flattening at higher doses would preclude cure and would suggest that "saturable passive resistance" (deficiency of factors required for cell killing) limits maximum achievable cell kill. An example of factors that could flatten the DRC at higher doses and lead to saturable passive resistance would be presence of quiescent, noncycling cells.

Key Words: Small cell, Dose-response, Resistance, Quiescence.

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Chemotherapy with or without radiotherapy for patients with limited disease (LD) small cell lung cancer (SCLC) has a 5-year survival rate of 10 to 25%.[1,2] In extensive disease (ED) SCLC, chemotherapy gives complete responses in more than 20% of patients and prolongs median survival.[1,2] Common regimens used in SCLC include etoposide-cisplatin (EP) and cyclophosphamide-doxorubicin-vincristine (CAV). Carboplatin, ifosfamide, epirubicin, paclitaxel, vinorelbine, topotecan, irinotecan, gemcitabine, pemetrexed, and other agents may also be used.[1–5] Patients with prior responses often benefit from further chemotherapy if progression occurs more than 3 months after completing prior chemotherapy ("sensitive relapse"), whereas they will generally be resistant to further chemotherapy if progression occurs during or rapidly after completing front-line chemotherapy ("refractory relapse").[6] Although the major factors underlying resistance to chemotherapy remain undefined, numerous factors may contribute.[7,8]

We postulated that dose-response curve (DRC) shape (log % cell survival versus dose) would reflect major resistance mechanisms limiting drug efficacy (Figure 1).[8,9] By this hypothesis, excess of a resistance factor such as an efflux pump and DNA repair ("active resistance") would give a DRC shoulder, analogous to competitive inhibition of drug effect. In this situation, the DRC slope would steepen after resistance factors had been overwhelmed by higher doses. One would not expect DRC flattening, because one would not expect the resistance mechanism to be ineffective at low doses and then become increasingly effective at higher doses. Mutation or alteration of a factor such as a target or drug uptake mechanism ("nonsaturable passive resistance") would give reduced DRC slope, pharmacodynamically analogous to decreased affinity of a drug for its receptor. The rationale for the explanation of nonsaturable passive resistance is that one would not expect the efficacy of a resistance factor to increase proportionately to the effect of an increasing drug dose. Saturation or deficiency of something required for cell killing (e.g., a drug uptake system or cells in a sensitive phase of the cell cycle) ("saturable passive resistance") would give DRC flattening at higher doses, analogous to noncompetitive inhibition of drug effect. In non-small cell lung cancer (NSCLC), we found that DRCs flatten at higher doses for all regimens assessed, suggesting that in NSCLC efficacy is limited by saturation or deficiency of factors required for drug effic-

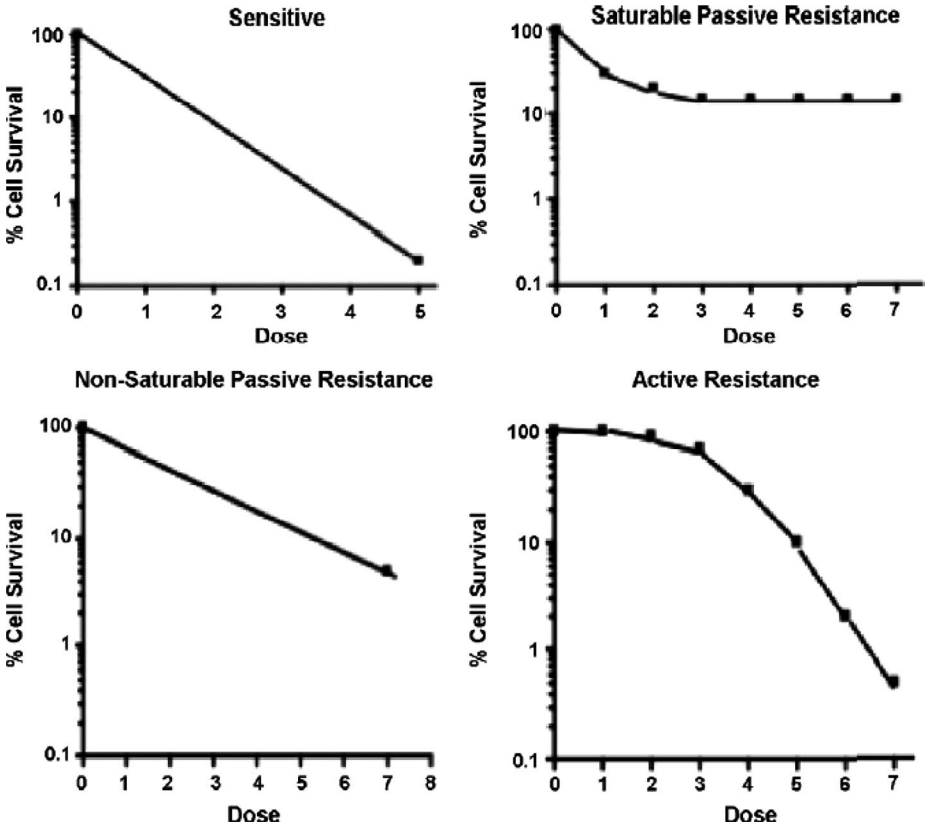

FIGURE 1. We postulated that dose-response curve shape would reflect whether resistance was due to deficiency or saturation of a factor required for drug effect (which would result in flattening of the dose-response curve at higher doses, "saturable passive resistance," analogous to noncompetitive inhibition of drug effect), due to mutation, etc., resulting in decreased affinity of a drug for an uptake system, target, etc. (which would give decreased curve slope, "nonsaturable passive resistance"), or due to excess of a resistance factor (which would give a shoulder on the dose-response curve if log effect is plotted against linear dose, "active resistance," analogous to competitive inhibition of drug effect). Reprinted with permission from *Crit Rev Oncol Hematol*.[8]

acy.[10] Herein, we used DRC assessments in SCLC as a basis for generating hypotheses regarding resistance mechanisms limiting drug efficacy in extensive disease.

METHODS

To be eligible for inclusion, a study had to report response rate using World Health Organization[11] or RECIST[12] criteria for front-line chemotherapy in ED SCLC. Studies were not included if they used alternating or cross-over designs, used radiotherapy, did not differentiate between ED and LD SCLC or between SCLC and NSCLC (if both LD and ED or both SCLC and NSCLC were included in the study), were restricted to tumor in a specific anatomic location (e.g., brain), or were testing primarily maintenance therapy or new drug administration methods or schedules. Using PUBMED and manual searches, we identified 46 studies (reporting 62 drug combinations) published between 1985 and 2005 that met all inclusion criteria (Table 1).[13–58]

We used response rate to roughly estimate mean % tumor cell kill for each trial. We assumed tumor cell kill was proportional to reduction in tumor volume, recognizing that several factors would reduce estimate accuracy, including tumor regrowth between cycles, residual necrotic or stromal cells, tumor measurement inaccuracies, tumor shape irregularity, etc., and recognizing that the relationship between response rate and % tumor cell kill has to our knowledge never actually been proven in either preclinical or clinical investigations. For complete responses (CR), we assumed more than 99% tumor cell kill. For a partial response, a tumor diameter decreases to less than 0.7 of the original diameter (with a decrease in the product of widest perpendicular diameters to $<0.7 \times 0.7 = 0.49$ of the original). This in turn translates roughly into a reduction in tumor volume to $<0.7 \times 0.7 \times 0.7 = 0.343$ of the original volume, such that the tumor has to have lost more than 65.7% of its original volume to be classified as a partial response. Hence, for the purposes of this study, we assumed that the average patient with a partial response would have an 83% reduction in tumor volume [(100 + 65.7)/2] and an average 83% cell kill. Because stable disease (SD) represents a 30% reduction in tumor diameter up to a 12 to 20% increase in tumor diameter (depending on whether World Health Organization or RECIST criteria are used), we assumed that the average SD patient had a 13% cell kill. For patients with progressive disease (PD), we assumed a 0% cell kill, recognizing that this would substantially underestimate cell kill in some patients, such as those who had initial tumor shrinkage followed by rapid regrowth. Mean % tumor cell kill for the average patient in an individual study was calculated as $100 \times ([\text{no. CR} \times 0.99] + [\text{no. PR} \times 0.83] + [\text{no. SD} \times 0.13])/\text{number of patients in the study}$.

Nonlinear meta-regression analysis (with each study weighted by number of patients) was then performed using SAS to model the relationship between mean % cell survival and dose intensity. Based on observations in our earlier studies in NSCLC,[10] we hypothesized that in SCLC we would initially see a downward slope on the survival curve with

TABLE 1. Regimens Included

Regimen	No. of Studies
Cisplatin + etoposide	9
+Ifosfamide	3
+Paclitaxel	2
+Irinotecan	2
+Topotecan	1
+Doxorubicin + vincristine	2
+Doxorubicin + cyclophosphamide	1
+Epirubicin + cyclophosphamide	2
+Epirubicin + ifosfamide	1
Cisplatin + epirubicin	1
Cisplatin + irinotecan	2
Cisplatin + paclitaxel + topotecan	1
Carboplatin + etoposide	3
+Vincristine	1
+Epirubicin	2
+Epirubicin + ifosfamide	1
Carboplatin + teniposide	1
Carboplatin + vinorelbine	2
Carboplatin + paclitaxel	1
+Ifosfamide	1
Cyclophosphamide + epirubicin	1
Cyclophosphamide + etoposide	2
Etoposide	1
Etoposide + ifosfamide	1
Etoposide + paclitaxel	1
Etoposide + vincristine	1
Docetaxel	1
Docetaxel + gemcitabine	2
Doxorubicin + etoposide + ifosfamide	1
Epirubicin	1
Epirubicin + ifosfamide + vindesine	3
Paclitaxel	2
Topotecan	1
Vinorelbine	1

increasing dose, followed by a flattening of the curve at highest doses. To test this hypothesis, we assessed whether data could be fit using a weighted nonlinear regression function characterized by the following mean function:

$$\%\ \text{Tumor Cell Survival} = \gamma(\beta+(1-\beta)\exp(\alpha \times \text{dose-intensity})),$$

subject to the restrictions $0 < \beta < 1$, $-5 < \alpha < 0$, $0 < \gamma < 100$, where the α parameter measures the relationship between dose and cell survival, β is the point at which cell survival plateaus, and γ is a scaling factor to permit scaling from 0 to 100%. Note that the α parameter characterizes the effect of dose on % cell survival and is set to be negative to ensure that % cell survival decreases with dose; the β parameter, as stated above, characterizes a level at which % cell survival plateaus and below which % cell survival does not fall. The plateau point characterized by β can be thought of as analogous to the cure rate fraction in some survival models.

TABLE 2. Number of Patients in Evaluable Studies

Drug	No. of Patients		
	Total No. of Patients (No. of Studies)	Study Size (Median)	Study Size (Range)
Cisplatin	2198 (30)	58	15–283
Cyclophosphamide	294 (8)	32	17–65
Carboplatin	605 (11)	43	28–156
Ifosfamide	588 (11)	54	16–87
Etoposide	2831 (40)	51	16–283
Epirubicin	680 (11)	65	30–78
Paclitaxel	575 (8)	42	34–283

Weighted linear regression analysis was used where data could not be fit to this nonlinear model. The criteria for using weighted linear regression models in place of weighted nonlinear models were when the weighted linear regression model resulted in lower (i.e., better) Akaike information criterion than the weighted nonlinear model. For carboplatin trials reporting dose as mg/m^2, AUC dose was estimated. For oral etoposide, 50% bioavailability was assumed.[59]

Analyses were done over the entire dose range available for each drug and were repeated after eliminating lower doses at or below the lower limit of those commonly used.

RESULTS

Sufficient data were available to permit analyses for cisplatin, carboplatin, cyclophosphamide, ifosfamide, etoposide, epirubicin, and paclitaxel (Table 2), whereas data were insufficient for analysis of other agents. Figure 2 shows the curves over the entire dose range assessed for each drug. Data were fit using a weighted nonlinear meta-regression model for cisplatin and cyclophosphamide, whereas weighted linear regression models are presented for the other agents, because the β parameter could not be fit by the nonlinear regression model. The parameter estimates and SEs for the models when all data are used for modeling are given in Table 3. In addition, for each model, we present the fitted models in Figures 2 and 3. For each of cisplatin, cyclophosphamide, ifosfamide, etoposide, and paclitaxel, there was a downward slope on the DRC, with increasing cell kill with increasing dose intensity, although this association between dose intensity and cell kill did not achieve statistical significance. When lowest drug doses were omitted (Figure 3), data for each of cisplatin, carboplatin, cyclophosphamide, and ifosfamide could be fit by the weighted nonlinear meta-regression model, and there was a downward curve slope for all seven agents, although again, statistical significance was not achieved.

Similar results were obtained using nonweighted analyses and using log % cell survival rather than % cell survival (data not shown). Irrespective of drug doses and combinations used, mean cell kill generally did not exceed 90% (Figures 2 and 3). Although several studies achieved this cell kill, none substantially exceeded it no matter how many drugs were used and no matter what doses per cycle or total cumulative doses were used.

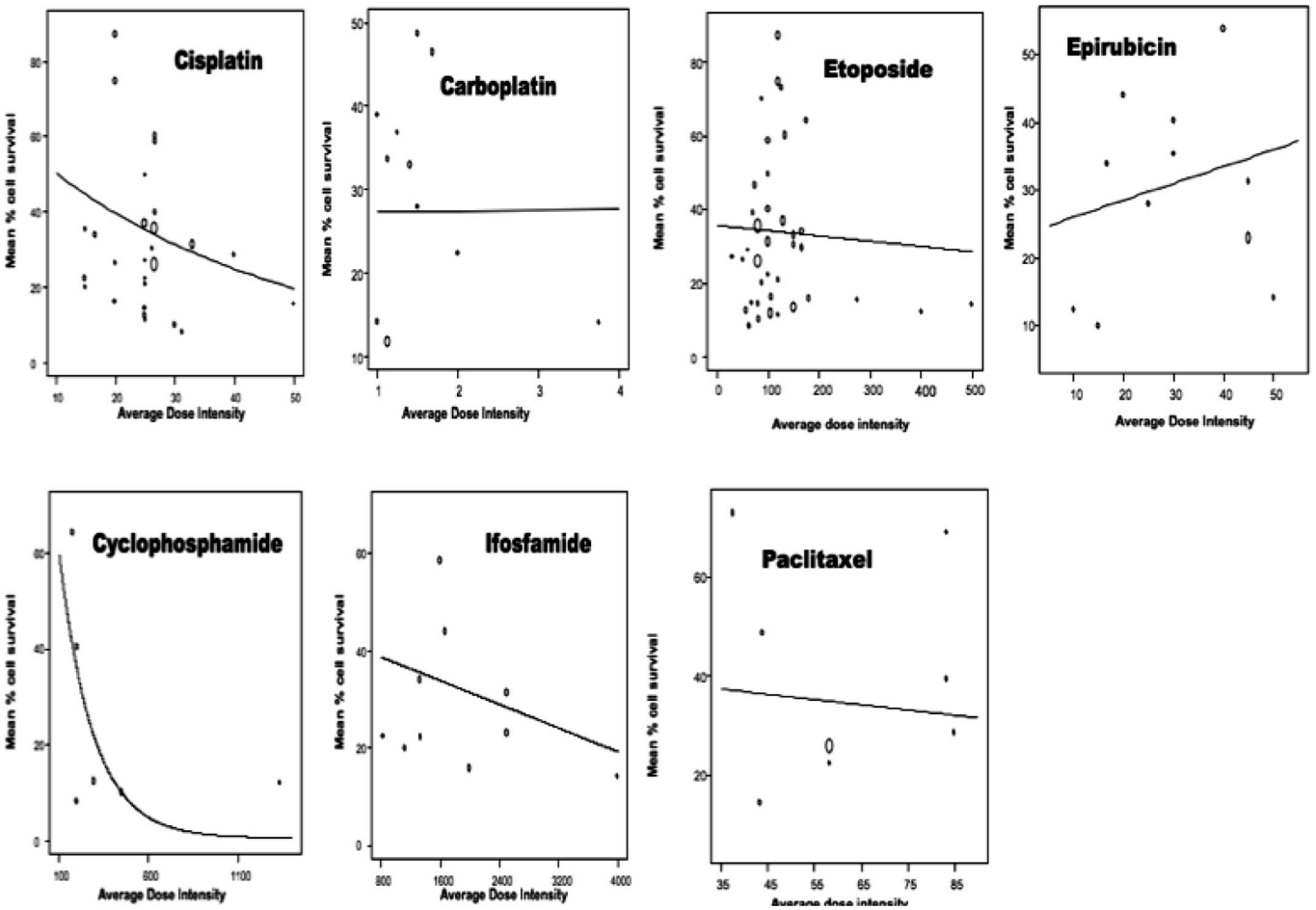

FIGURE 2. Weighted nonlinear meta-regression analysis (where the α parameter measures the relationship between dose and cell survival) or weighted linear regression analysis (where the β parameter measures the relationship between dose and cell survival) over the entire dose range assessed. Data for each of cisplatin ($n = 30$, $\alpha = -0.02$) and cyclophosphamide ($n = 8$, $\alpha = -0.01$) could be fitted to the nonlinear regression model, whereas data for each of etoposide ($n = 40$, $\beta = -0.01$), carboplatin ($n = 11$, $\beta = 0.14$), epirubicin ($n = 11$, $\beta = 0.25$), paclitaxel ($n = 8$, $\beta = -0.11$), and ifosfamide ($n = 11$, $\beta = -0.01$) could not be fitted to the nonlinear regression model and were instead assessed by linear regression analysis.

DISCUSSION

Despite often initially responding well to chemotherapy, ED SCLC remains incurable. The reasons for this incurability are unknown. Several resistance mechanisms have been described in SCLC cell lines and xenografts,[8,60] but it is unclear which of these are important clinically. Although this study was limited by a relatively small number of evaluable studies and by our inability to correct for the contribution of concurrent agents in drug combination studies, the lack of a statistically significant association between dose intensity of any agent and estimated cell kill would argue against a steep DRC for any of the agents assessed. This is in keeping with our observation that there seems to be a maximum mean cell kill of around 90% across all ED SCLC studies, irrespective of drug type, number of drugs used, drug doses per cycle, dose intensity, total cumulative drug dose administered, treatment schedule, or cell cycle specificity of the agents used. This apparent maximum cell kill of around 90% irrespective of drug used would suggest that DRCs for all agents flatten at higher drug doses, and that there is no combination, dose, or schedule of currently available agents that would be capable of curing ED SCLC. Even very high chemotherapy doses failed to improve outcome. This is similar to our observation of DRC flattening at higher doses in NSCLC[10] and is in keeping with the inability of even very high drug doses with bone marrow transplantation to cure any metastatic epithelial malignancies.[61] Overall, the observations in this study are in keeping with our earlier conclusion[10] that there could possibly be a common factor that limits curability of all metastatic epithelial malignancies. If interpreted according to our hypothesis on the link between DRC shape and resistance mechanisms,[9] this DRC flattening would suggest that the major problem is one of deficiency or saturation of a factor required for drug efficacy, rather than incurability being due to excess of resistance factors such as efflux pumps or DNA repair pathways. By our hypothesis,[9] "active" resistance factors such as efflux pumps or DNA repair pathways would be most important at low drug doses

TABLE 3. Model Estimates, SEs, and 95% Confidence Intervals

Treatment	Model	Parameter	Parameter Estimate (SE)	Lower 95% Limit	Upper 95% Limit
Cisplatin	Nonlinear	α	−0.0233 (0.02)	−0.0624	0.0159
		β	0.00 (0.00)	0.000	0.000
		γ	63.36 (29.65)	2.63	124.10
Cyclophosphamide	Nonlinear	α	−0.0052 (0.003)	−0.011	0.00084
		β	0.0061 (0.200)	−0.484	0.4958
		γ	100.00 (0.00)	100.000	100.000
Carboplatin	Linear	Intercept	27.179 (11.730)	0.645	53.714
		Slope	0.137 (7.527)	−16.889	17.164
Ifosfamide	Linear	Intercept	43.636 (12.536)	15.277	71.994
		Slope	−0.006 (0.006)	−0.020	0.008
Etoposide	Linear	Intercept	35.671 (7.311)	20.871	50.471
		Slope	−0.014 (0.056)	−0.128	0.100
Epirubicin	Linear	Intercept	23.584 (10.728)	−0.685	46.754
		Slope	0.248 (0.334)	−0.507	1.003
Paclitaxel	Linear	Intercept	41.072 (30.349)	−33.189	115.332
		Slope	−0.105 (0.499)	−1.326	1.115

but should be surmountable by increasing drug doses. Although there was a downward slope on many of the DRCs, this was not statistically significant for any agent, and the observation of a maximum mean cell kill that was consistent across all agents suggests that there is an upper limit to the cell kill achievable by augmenting drug doses.

One possible explanation of the DRC flattening would be the presence of quiescent cells in the tumor that survive the first cycle of chemotherapy, irrespective of drug dose. Quiescent cells generally have increased resistance to therapy,[62–64] and various preclinical observations support a link between quiescence and resistance. For example, resistance may be accompanied by decreased tumorigenic capacity,[65] a decrease in uptake across cell membranes of a broad range of factors and drugs,[66] downregulation of expression of various membrane receptors and transporters,[66] decreased amount and altered localization of a variety of membrane proteins,[65] faulty or decreased endocytosis,[66,67] reversible senescence,[68,69] autophagy,[70] or appearance of nondividing multinucleated cells that may later resume cell cycling.[71]

If quiescent cells did survive the first cycle of chemotherapy irrespective of dose (thereby flattening DRCs), these surviving quiescent cells might maintain their quiescent phenotype for a prolonged period of time after drug exposure but might ultimately start cycling again, as has been described for quiescent cells in some preclinical lung cancer systems.[68,69,71] The fact that occasional late relapses are seen in patients with SCLC[72–75] is in keeping with SCLC cells being capable of reversible quiescence. If resistance was due to quiescence, then the tumor cells might potentially be sensitive again when they ultimately resumed cycling. Such a pattern of prolonged quiescence followed by drug-sensitive tumor regrowth would be in keeping with the observation in SCLC that patients who do not require reinitiation of chemotherapy until more than 3 months after their prior therapy may again be sensitive to chemotherapy when they ultimately do suffer tumor regrowth (the "sensitive relapse" phenotype[6]). In addition, with increasing time after exposure of cancers to prior therapy, there can be reduction in DNA hypermethylation and re-expression of transporters and other factors that have been downregulated as a consequence of exposure to chemotherapy and that may be required for drug efficacy.[76]

Conversely, chemotherapy exposure can also in some cases result in recruitment of quiescent cells into active proliferation,[77] and there can be rapid regrowth of tumors between chemotherapy cycles, with increasingly rapid repopulation and resistance due to this "accelerated repopulation."[78] Exposure of tumor cells to chemotherapy can also result in upregulation of expression of resistance factors.[8,79,80] Tumors that regrow during chemotherapy or shortly after completion of chemotherapy are generally resistant to further chemotherapy,[6] and this could potentially be attributable to the combination of accelerated repopulation[78] and upregulation of expression of resistance factors.[8,79,80] In this instance, increasing drug doses might hypothetically somewhat improve therapy efficacy, but residual resistant quiescent cells would still ultimately limit outcome and flatten DRCs at highest doses.

Although the above could explain both DRC flattening and relapse patterns, it is stressed that our proposed explanation is highly speculative. In both preclinical and clinical studies, numerous resistance mechanisms have been noted in SCLC,[8,60] and it remains unknown which ones are most important clinically. The field would benefit from carefully done translational studies (with tumor biopsies before therapy initiation and again at the time of maximum response and at the time of relapse) to assess how tumor cell growth characteristics, resistance factors, and factors required for therapy efficacy (e.g., drug obligate targets, drug transporters, drug activators, and proapoptotic factors) change over the course of therapy. If the initial presence of quiescent cells did prove to be central to the ultimate failure of therapy, then it would be particularly important to define potentially exploitable targets in these quiescent cells.

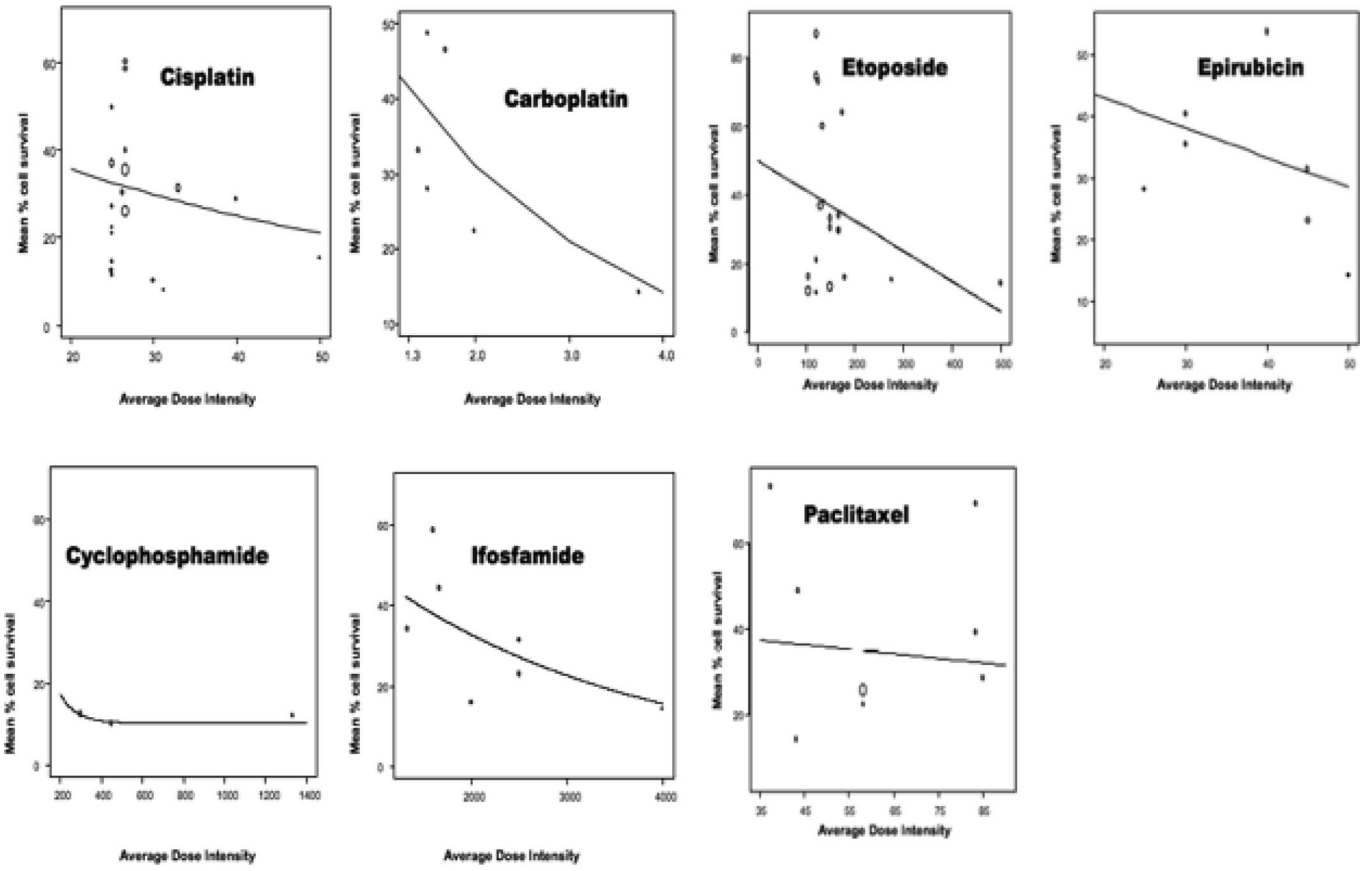

FIGURE 3. Weighted nonlinear meta-regression analysis (where the α parameter measures the relationship between dose and cell survival) or weighted linear regression analysis (where the β parameter measures the relationship between dose and cell survival) over higher doses (with elimination of data for doses of etoposide ≤100, cisplatin ≤20, epirubicin ≤20, cyclophosphamide ≤200, paclitaxel ≤35, or ifosfamide ≤1250 mg/m^2/wk or carboplatin ≤ AUC 1.25/wk). Data for each of cisplatin ($n = 22$, $\alpha = -0.02$), carboplatin ($n = 6$, $\alpha = -0.39$), cyclophosphamide ($n = 3$, $\alpha = -0.01$), and ifosfamide ($n = 9$, $\alpha = -0.0004$) could be fitted to the nonlinear regression model, whereas data for each of etoposide ($n = 20$, $\beta = -0.09$), epirubicin ($n = 7$, $\beta = -0.48$), and paclitaxel ($n = 8$, $\beta = -0.11$) could not be fitted to the nonlinear regression model and were instead assessed by linear regression analysis.

The main purpose of this assessment was not to determine whether or not there is a benefit from administering higher doses of chemotherapy but rather to assess DRC shape, and the relatively small number of evaluable studies meant that statistical power was low. We did observe a downward slope in the DRCs for five of the seven agents over all dose intensities, and a downward slope was also seen for the two remaining drugs if the relatively flat early low-dose curve portion was omitted, but as noted above, the association between dose intensity and efficacy did not achieve statistical significance for any of the agents assessed. Recent reviews have also concluded that there is no proven benefit of higher dose intensity or dose density in SCLC.[1,2,4,5,81] Nevertheless, available data suggest that it may be premature to conclude that dose is largely unimportant. In randomized trials, significant improvements in median survival,[37,82–89] % 2-year survival,[37,82,83,85–88] relapse-free survival,[90] complete response rates[84,85,90–92] or overall response rates,[52,83,87,89,93–95] or a statistically nonsignificant trend toward higher response rates[52,82,84,86,93,96] or longer survival[90,97] have been reported with a variety of higher dose approaches or with addition of more agents to a therapy regimen. Only a minority of randomized trials have failed to show even a trend toward improvement in either survival or response with higher dose approaches or with increase in the number of agents used.[45,48,98–103] Hence, despite the fact that even high drug doses are incapable of curing ED SCLC, it remains possible that higher drug doses may at least modestly improve palliation and short-term tumor control.

In summary, while maintaining relatively high doses of chemotherapy may possibly have a modestly positive impact in ED SCLC, DRC flattening at higher doses may preclude cure with currently available agents. The apparent DRC flattening would suggest that incurability of ED SCLC is due to deficiency or saturation of a factor or factors required for drug efficacy, and further progress will depend on our defining new therapeutic methods specifically targeting these resistant cells.

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